OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Endo-free Plasmid DNA Giga Kit

D6234-00	2 preps
D6234-01	5 preps

August 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Endo-free Plasmid DNA Giga Kit

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Introduction

The E.Z.N.A.® family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.® Endo-Free Plasmid DNA Giga Kit combines time-tested consistency of alkaline-SDS lysis of bacterial cells with Omega Bio-tek's innovative high-efficiency DNA binding technology to recover large scale, high-quality plasmid DNA. This method facilitates the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit uses a syringe-format system that is designed to work in combination with the centrifugation steps following the alkaline lysis of bacterial cells. The Lysate Clearance Filter Syringe completely removes SDS precipitates and clears bacterial lysates in a fraction of the time required by centrifugation alone.

Yields vary according to plasmid copy number, E.coli strain, and growth conditions, but 1L overnight culture in LB medium typically produces 5-10 mg high copy plasmid DNA. Up to 1 liter overnight culture may be processed when working with low copy number plasmids. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

New In this Edition:

- The latest edition of this manual has been redesigned to enhance readability and protocol quality.

Kit Contents

Product Number	D6234-00	D6234-01
Preparations	2	5
HiBind® DNA Giga Columns	2	5
Solution I	270 mL	3 x 230 mL
Solution II	270 mL	3 x 230 mL
Neutralization Buffer	270 mL	3 x 230 mL
PFC Binding Buffer	270 mL	3 x 230 mL
EWR Wash Buffer	90 mL	250 mL
ETR Binding Beads	35 mL	80 mL
DNA Wash Buffer	50 mL	2 x 50 mL
Endotoxin-free Water	50 mL	100 mL
RNase A	1 mL	3 x 0.7 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® Endo-free Plasmid DNA Giga Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Store ETR Binding Beads at 2-8°C. Store RNase A at 2-8°C. Store Solution I/RNase A at 2-8°C after being combined (see Page 7). All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in Solution II and PFC Binding Buffer. Dissolve such deposits by warming the solution at 50°C and gently shaking.

Guidelines for Vacuum Manifold

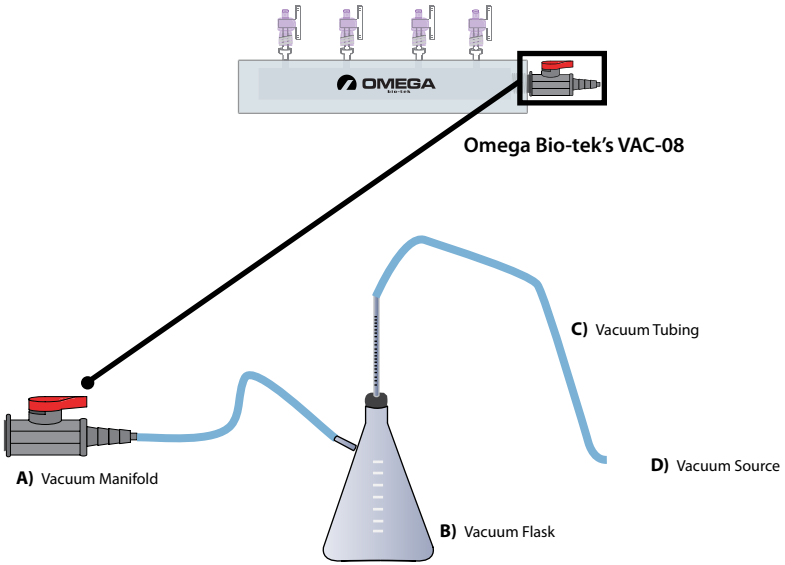
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α™ , DH1, and C600. These host strains yield high-quality DNA with E.Z.N.A.® Endo-free Plasmid DNA Giga Kit Protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high-quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, Solution II, and Neutralization Buffer, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The E.Z.N.A.® Endo-free Plasmid DNA Giga Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the HiBind® DNA Giga Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Preparing Reagents

- Add vial of RNase A to the bottle of Solution I provided and store at 2-8˚C.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6234-00	200 mL
D6234-01	200 mL per bottle

- Prepare ETR Binding Beads as follows:

1. Aliquot the required amount of ETR Binding Beads. Each prep will require 15 mL ETR Binding Beads. Prepare within 4 hours of processing.
2. Centrifuge at 3,000 x *g* for 3 minutes.
3. Aspirate and discard the supernatant.
4. Add 2 volumes water (not provided).
5. Vortex to resuspend the ETR Binding Beads.
6. Repeat Steps 2-5 for a second water wash.
7. Centrifuge at 3,000 x *g* for 3 minutes.
8. Aspirate and discard the supernatant.
9. Add 1 volume water (not provided).
10. Vortex to resuspend the ETR Binding Beads.

E.Z.N.A.® Plasmid DNA Giga Kit - Vacuum Protocol

This Protocol is designed to isolate 10 mg of high Copy-Number plasmids or 1 mg of low copy-Number Plasmids from 1 L overnight cultures using the E.Z.N.A.® Endo-Free Plasmid Giga Kit. Binding more than 10 mg of Plasmid DNA to the HiBind DNA Giga Column can increase processing time and cause the column to clog.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Centrifuge with swing bucket rotor capable of 5,000 x *g* with adapters for 50 mL centrifuge tubes and 500 mL centrifuge bottles
- Vacuum pump capable of generating -200 to -600 mbar
- Vacuum manifold (Cat No. VAC-08)
- 50 mL centrifuge tubes
- 500 mL centrifuge bottles (i.e. Nalgene 3120)
- Vortexer
- Ice bucket

Before Starting:

- Chill Neutralization Buffer on ice
- Prepare ETR Binding Beads, DNA Wash Buffer, and Solution I according to the "Preparing Reagents" section on Page 7

1. Inoculate 2 L LB/ampicillin (50 µg/mL) medium placed in a 5 liter culture flask with *E. coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 hours. It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

   Note: Optimal growth conditions of bacteria is vital of obtaining maximal plasmid DNA yields. The best conditions are achieved by picking a single colony from a freshly transformed or freshly grown plate to inoculate a 2-5 mL starter culture containing the appropriate antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm). Using a flask or vessel with a volume of a least 3-4 times the volume of the culture, dilute the starter culture 1/500 to 1/1000 times into warm culture media containing antibiotics. Grow at 37°C for 12-16 hr with vigorous shaking (~300 rpm).

   Following overnight bacterial growth, an OD_{600} of 1.5~2.0 indicates a well-grown culture. For the best result determination of OD_{600} for each culture is recommended. It is important to dilute the bacterial culture (10 to 20 fold) to enable photometric measurement in the linear range between 0.1 and 0.5 OD_{600}. We recommend a bacterial density of between 2.0 and 3.0 at OD_{600}. When using nutrient-rich media, care should be taken ensure that the cell density does not exceed an OD_{600} of 3.0. If using a frozen glycerol stock as the inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then pick a single colony and inoculate the 2-5 mL starter culture as described above.

2. Centrifuge at 5,000 × *g* for 10 minutes at room temperature to pellet bacteria.

3. Decant or aspirate the media and discard. To ensure that all traces of media are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 125 mL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the "Preparing Reagents" section on Page 7.

5. Add 125 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. Let sit at room temperature for 3 minutes.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 125 mL ice-cold Neutralization Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

   Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Centrifuge at 4,000 x *g* for 20 minutes.

   Note: Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell debris. After centrifugation, a tightly packed pellet indicates efficient lysis.

8. Transfer the supernatant into a new 500 mL centrifuge bottle.

   Note: After the centrifugation, the supernatant should appear clear. If any precipitates are present in the supernatant, pass the sample through a filter paper such as Miracloth™ or a coffee filter before continuing.

9. Add 15 mL ETR Binding Beads. Vortex for 10-20 seconds to mix thoroughly.

   Note: ETR Binding Beads must be prepared before use. Please see the instructions in the "Preparing Reagents" section on Page 7.

10. Let sit for 5 minutes at room temperature.

11. Centrifuge at 4,000 x *g* for 10 minutes.

12. Carefully transfer the cleared supernatant to a new 500 mL centrifuge bottle. Avoid transferring the ETR Binding Bead pellet.

    Note: The ETR Binding Beads contains high levels of endotoxins and should not be transferred to the new vessel.

13. Measure the volume of the supernatant.

14. Add 1/3 volume PFC Binding Buffer. Vortex to mix thoroughly.

15. Prepare the vacuum manifold by following the manufacturer's instructions.

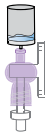
16. Remove and save the plunger and the tip cap from the HiBind® DNA Giga Column.

    Note: DO NOT discard the plunger or tip cap. They will be required later.

17. Connect the HiBind® DNA Giga Column to the vacuum manifold. Refer to the Illustrated Vacuum Setup on Page 4 for details.

18. Transfer the sample from Step 14 by CAREFULLY decanting it into the HiBind® DNA Giga Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Giga Column.

    Note: Do not allow the HiBind® DNA Giga Column to become empty until you have transferred all of the sample from Step 14.



Leaving ~1 mL solution in the column during Step 18 reduces foaming and clogging which decreases total processing time and improves efficiency.

19. Add 40 mL EWR Wash Buffer to the HiBind® DNA Giga Column.

20. Turn on the vacuum source to draw the buffer through the column.

21. Turn off the vacuum.

22. Add 50 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 7.

23. Turn on the vacuum source to draw the buffer through the column.

24. Turn off the vacuum.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

26. Continue to apply the vacuum to the HiBind® DNA Giga Column for 15 minutes.

    Note: It is important to dry the HiBind® DNA Giga Column matrix before elution. Residual ethanol may interfere with downstream applications.

27. Turn off the vacuum.

28. Remove the HiBind® DNA Giga Column from the vacuum manifold.

29. Place the tip cap (removed in Step 16) firmly on the HiBind® DNA Giga Column.

30. Add 10-15 mL Endotoxin-free Water directly to the center of the column matrix.

31. Let sit for 5 minutes at room temperature.

32. Remove the tip cap and hold the HiBind® DNA Giga Column over a 50 mL centrifuge tube (not provided).

33. Gently insert the plunger to expel the Endotoxin-free Water into the 50 mL centrifuge tube.

34. Repeat Steps 30-33 for a second elution step.

    Note: To maintain a higher concentration of plasmid DNA, the first eluate can be reused.

35. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Low DNA Yields	
Poor cell lysis	• Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually. • Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to vortex cell suspension to completely disperse bacterial cells. • Solution II if not tightly closed, may need to be replaced.
Bacterial colony is overgrown or not fresh	Do not incubate cultures for more than 24 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	If using Endotoxin-free Water for elution, adjust the pH of the water to pH 8.0
Alkaline lysis was prolonged	Reduce the lysis time (Solution II) to 2 minutes or until the suspended cells form a clear viscous solution
Too many or too few cells were used	Confirm the cell density by measuring OD . To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm.
DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to the instructions on Page 7.
High molecular weight DNA contamination of product	
Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

Plasmid DNA floats out of well while loading agarose gel	
Ethanol has not been removed completely from column following wash steps	Dry the column as instructed before elution. Incubate columns for 10 minutes at 65°C to completely dry membrane after vacuum step for drying.
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260} /A_{280} ratio is high or low).	
DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that RNase A was added to Solution I prior to first use. The RNase A Solution may degrade due to high temperatures (>65ºC) or prolonged storage (> 6 months) at room temperature).
Background reading is high due to silica fines	Centrifuge the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purification is incomplete due to column overloading	Reduce the initial volume of culture.
Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 800-832-8896)

Product	Part Number
Vacuum Manifold	VAC-08
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
Neutralization Buffer (250 mL)	PS004
DNA Wash Buffer Concentrate (100 mL)	PS010
RNase A (5 mL)	RNA-03

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

Notes: